



**University of  
Zurich**<sup>UZH</sup>

**Zurich Open Repository and  
Archive**

University of Zurich  
University Library  
Strickhofstrasse 39  
CH-8057 Zurich  
[www.zora.uzh.ch](http://www.zora.uzh.ch)

---

Year: 2014

---

## **Balgacyclamides, Antiplasmodial Heterocyclic Peptides from *Microcystis aeruginosa* EAWAG 251**

Portmann, Cyril ; Sieber, Simon ; Wirthensohn, Silvan ; Blom, Judith F ; Da Silva, Laeticia ; Baudat, Emilie ; Kaiser, Marcel ; Brun, Reto ; Gademann, Karl

**Abstract:** The isolation and structural characterization of three new heterocyclic and macrocyclic peptides, balgacyclamides A-C, from *Microcystis aeruginosa* EAWAG 251 are reported. The constitutions were determined by 2D-NMR methods and mass spectrometry, and the configurations were assigned after ozonolysis and hydrolysis by HPLC-MS methods using Marfey's method as well as GC-MS using authentic standards. Balgacyclamides A and B were active against *Plasmodium falciparum* K1 in the low micromolar range, while displaying low toxicity to rat myoblasts.

DOI: <https://doi.org/10.1021/np400814w>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-107280>

Journal Article

Accepted Version

Originally published at:

Portmann, Cyril; Sieber, Simon; Wirthensohn, Silvan; Blom, Judith F; Da Silva, Laeticia; Baudat, Emilie; Kaiser, Marcel; Brun, Reto; Gademann, Karl (2014). Balgacyclamides, Antiplasmodial Heterocyclic Peptides from *Microcystis aeruginosa* EAWAG 251. *Journal of natural products*, 77(3):557-562.

DOI: <https://doi.org/10.1021/np400814w>

# Balgacyclamides, Antiplasmodial Heterocyclic Peptides from *Microcystis aeruginosa* EAWAG 251

*Dedicated to Prof. Otto Sticher for his pioneering work in  
pharmacognosy and phytochemistry*

*Cyril Portmann,<sup>°</sup> Simon Sieber,<sup>†</sup> Silvan Wirthensohn,<sup>†</sup> Judith F. Blom,<sup>‡</sup> Laeticia Da Silva,<sup>°</sup> Emilie  
Baudat,<sup>°</sup> Marcel Kaiser,<sup>§</sup> Reto Brun<sup>§</sup> and Karl Gademann<sup>°, †, \*</sup>*

Chemical Synthesis Laboratory, SB-ISIC-LSYNC, Swiss Federal Institute of Technology (EPFL),  
CH-1015 Lausanne, Switzerland

Department of Chemistry, University of Basel, St. Johannis-Ring 19, CH-4056 Basel, Switzerland

Limnological Station, Institute of Plant Biology, University of Zürich,  
CH-8802 Kilchberg, Switzerland

Parasite Chemotherapy, Swiss Tropical and Public Health Institute (Swiss TPH), CH-4002 Basel,  
Switzerland

\* To whom correspondence should be addressed. Tel: ++41 61 267 11 44. Fax: ++41 61 267 11  
45. E-mail: karl.gademann@unibas.ch

<sup>°</sup> Swiss Federal Institute of Technology

<sup>†</sup> University of Basel

<sup>‡</sup> University of Zürich

<sup>§</sup> Swiss Tropical and Public Health Institute

## ABSTRACT

The isolation and structural characterization of three new heterocyclic and macrocyclic peptides, balgacyclamides A–C from *Microcystis aeruginosa* EAWAG 251 is reported. The constitution was determined by 2D-NMR methods and mass spectrometry and the configuration was assigned by HPLC-MS methods using Marfey's method as well as GC-MS using authentic standards. Balgacyclamides A and B were active against *Plasmodium falciparum* K1 in the low micromolar range, while displaying low toxicity to rat myoblasts.

## INTRODUCTION

Cyclic peptides and derived macrocycles have recently received significant interest in a variety of fields, ranging from biosynthesis studies to drug discovery and development<sup>1</sup>. The potentially large, but structurally often well defined conformational space sampled, combined with the variety of amino acids renders peptides ideally suited to interact with many receptors or to interfere with protein/protein interactions.<sup>1</sup> Cyanobactins constitute a particular subset of cyclic peptides produced by cyanobacteria and can be defined as cyclic, ribosomally produced peptides (RiPP)<sup>2</sup> containing heterocyclic modifications.<sup>3</sup> They have been found not only in the producing organism, but also in other animals where they have been suggested to be of symbiotic or dietary origin.<sup>4</sup> A particular subset, the cyclamides are structurally well defined macrocyclic hexapeptides, where often hydrophobic residues flanked by heterocyclic rings such as oxazol(ine) or thiazol(ine) residues are observed.<sup>5</sup> While their biosynthesis has been investigated,<sup>2–4,5m</sup> the bioactivity of these compounds both in their ecological niche as well as in a therapeutic setting has received much less attention. Early assumptions on the bioactivity suggested a role as allelochemicals, which has been supported by algicidal effects.<sup>6</sup> Another seemingly unrelated activity is related to multidrug-resistance reversing activity, which has been reported for several members of this family.<sup>7</sup> Most interestingly, Gerwick and coworkers reported significant antimalarial activity for the venturamides.<sup>8</sup> Independently, we have determined antimalarial activity for the aerucyclamides<sup>9</sup> that displayed improved activity when compared to the earlier study.<sup>8</sup> The best compound in this series, aerucyclamide B displayed antimalarial IC<sub>50</sub> of 0.7  $\mu$ M against the *Plasmodium falciparum* K1 strain with a high selectivity over the L6 rat myoblasts cell line. Aerucyclamide B was taken up as a lead structure for a medicinal chemistry follow up program by Serra and coworkers that build on the first successful total synthesis of this compound.<sup>10</sup> Whereas these authors were able to briefly delineate a first structure activity relationship study, currently, the role of the different units on activity remained unclear. The current main hypothesis suggests that the degree of unsaturation of the heterocyclic rings is correlated with biological activity,<sup>8–10</sup> with higher unsaturation leading to more potent compounds. While this hypothesis is supported by several compounds (e.g.

oxidation of an thiazoline to an thiazole in aerucyclamide A and B led to 10 times increased activity), more compounds are needed to corroborate these observations. In this study, we report on the isolation of new cyclamides from cyanobacteria, and evaluate the antimalarial properties in the *P. falciparum* K1 assay.

## RESULTS AND DISCUSSION

The balgacyclamides were isolated from aqueous methanolic extracts of *Microcystis aeruginosa* EAWAG 251 (identical to PCC 7820). These compounds were purified by a combination of C<sub>18</sub>-SPE, semi-preparative and analytical reverse phase HPLC methods. The structure determination proved to be challenging due to the low isolation yield of 0.37 mg, 0.10 mg, and 0.53 mg per 10 liters of culture, for the balgacyclamides A (**1**), B (**2**) and C (**3**) respectively.

**Balgacyclamide A (1)** displayed an exact mass of  $m/z$  533.2552, which supports the molecular formula C<sub>25</sub>H<sub>37</sub>N<sub>6</sub>O<sub>5</sub>S (calcd 533.2546) for the [M+H]<sup>+</sup> pseudomolecular ion. The <sup>1</sup>H spectrum (DMSO-*d*<sub>6</sub>, 500MHz) displayed the typical pattern of a peptide (NMR data in table 1). Three NH resonances were present as doublet. Based on characteristic signals and COSY correlations, the fragments NH-Ala, NH-Val and NH-Ile were assigned. HSQC and HMBC experiments were used to assign the <sup>13</sup>C chemical shifts. Furthermore, two Thr residues missing the NH resonance were assigned as methyloxazoline (MeOzn) moieties. The last fragment was assigned as a thiazole (Tzl) based on its characteristic signals (H-18 Tzl, singlet,  $\delta_H$  8.30 ppm, C-18 Tzl  $\delta_C$  126.4 ppm).<sup>11</sup> The sequence of the amino acids was challenging to determine because of the presence of resonance overlap in the <sup>1</sup>H and <sup>13</sup>C spectra. HMBC long-range correlations were measured in two different solvents, DMSO-*d*<sub>6</sub> and CDCl<sub>3</sub>, to establish the sequence (NMR data in CDCl<sub>3</sub> are available in the supporting information). From the HMBC spectrum in DMSO-*d*<sub>6</sub>, correlations from H-2 of MeOzn1 to C-5 of Val and from NH-6 of Ile to C-1 of MeOzn1 were unambiguous. Additionally, a long range *J* coupling of 2.0 Hz was observed between the two C( $\alpha$ )-H of the MeOzn1 and of the Val residue. These long range *J* couplings are frequently found in cyclamides with oxazoline ring system.<sup>9,12</sup> From the HMBC spectrum recorded in CDCl<sub>3</sub>, correlation from NH-2 of Val to C-9 of MeOzn2 and from H-18 of Tzl to C-19 of Ile allowed to establish the partial sequence -Tzl-Ile-MeOzn1-Val-MeOzn2- (figure 1). Only the position of Ala was not established with the NMR data. Based on the molecular formula, balgacyclamide A (**1**) required to be teracyclic, therefore the peptide has to be a macrocycle. Therefore, the sequence of the peptide was assigned as cyclo-(-MeOzn1-Val-MeOzn2-Ala-Tzl-Ile-)-.

**Balgacyclamide B (2)** displayed an exact mass of  $m/z$  551.2657, which supports the molecular formula C<sub>25</sub>H<sub>39</sub>N<sub>6</sub>O<sub>6</sub>S (calcd 551.2652) for the [M+H]<sup>+</sup> pseudomolecular ion. Its molecular formula suggests that **2** is a H<sub>2</sub>O adduct of balgacyclamide A (**1**). The constitution of **2** was

obtained utilizing the same methods as for balgacyclamide A (**1**). As for **1**, NH-Ala, NH-Val and NH-Ile were identified, but instead of two MeOzn in **1**, one MeOzn and one NH-Thr were presents accounting for the difference in the molecular formula. The sequence was established by HMBC long-range correlations (figure 1). Correlations were observed from H-2 of MeOzn to C-5 of Val, from H-6 of Val to C-9 of Thr, from H-10 of Thr to C-13 of Ala, from H-14 of Ala to C-16 of Tzl, from H-18 of Tzl to C-19 of Ile and from H-20 of Ile to C-1 of MeOzn. As the  $^{13}\text{C}$  chemical shift of two carbonyls and the thioimide (why thioamide if it is MeOzn???) were close from each other (C-1 MeOzn  $\delta_{\text{C}}$  169.7, C-9 Thr  $\delta_{\text{C}}$  169.4 and C-19 Ile  $\delta_{\text{C}}$  169.6), the sequence was confirmed with ROESY correlations. Correlations were observed between H-4 of MeOzn and H-6 of Val, between NH-2 of Val and H-10 and H-11 of Thr, between NH-3 of Thr and H-14 and H-15 of Ala and between NH-6 of Ile and H-2 of MeOzn. As for cyclamide **1**, a long range  $J$  coupling of 1.6 Hz was observed between the two  $\text{C}(\alpha)\text{-H}$  of the MeOzn and of the Val residue. Only one link between C-16 and C-17 of Tzl could not be established with the different NMR experiments. Again, according to the molecular formula, balgacyclamide B (**2**) was required to be tricyclic and therefore C-16 and C-17 needed to be connected, thus closing the macrocyclic ring of cyclo-(-MeOzn-Val-Thr-Ala-Tzl-Ile-)-.

**Balgacyclamide C (3)** displayed an exact mass of  $m/z$  585.2512, which supports the molecular formula  $\text{C}_{28}\text{H}_{37}\text{N}_6\text{O}_6\text{S}$  (calcd 585.2495) for the  $[\text{M}+\text{H}]^+$  pseudomolecular ion. The six fragments MeOzn, NH-Phe, NH-Thr, NH-Gly, Tzl and NH-Ile were identified as described above. HMBC spectroscopy allowed the establishment of the sequence through a series of long-range correlations: from H-2 of MeOzn to C-5 of Phe, from H-6 of Phe to C-12 of Thr, from NH-3 of Thr to C-16 of Gly, from H-17 of Gly to C-18 of Tzl, from H-20 of Tzl to C-21 of Ile and from NH-6 of Ile to C-1 of MeOzn, which established the sequence as cyclo-(-MeOzn-Phe-Thr-Gly-Tzl-Ile-)-.

**The configuration of the balgacyclamides A-C (1-3)** was determined with the same strategy as for the aerucyclamides.<sup>9</sup> First, the compounds were ozonized to avoid the epimerization of the Ile residues that are next to the thiazole residues, as it is known that amino acids next to thiazole or oxazole residues are prone to epimerization during hydrolysis.<sup>13</sup> The compounds were then hydrolyzed, and half the amount of the hydrolyzed compounds was derivatized with the Marfey's reagent<sup>14</sup> while the other half was derivatized with trifluoroacetic acid anhydride. The Marfey's derivatives were analyzed by HPLC-MS to establish the configuration of the Ala, Thr, Val and Phe amino acids by comparing the retention time with authentic derivatized amino acids as standards and co-injection of authentic and natural samples was realized when necessary. The TFA derivatives were analyzed by GC-MS to establish the configuration of the Ile amino acids. It was necessary to perform a co-injection analysis with standard derived Ile to differentiate between the D-Ile and D-*allo*-Ile. This methodology established the presence of D-

Ala, L-Thr, L-Val and D-*allo*-Ile for the balgacyclamides A and B (**1** and **2**) and L-Thr, L-Phe, D-*allo*-Ile for balgacyclamide C (**3**). These experiments therefore established the structures of the balgacyclamides A-C as shown for compounds **1-3** (Figure 1)

The balgacyclamides A (**1**) and B (**2**) were evaluated for their antiparasite activity. Compounds **1** and **2** displayed micromolar IC<sub>50</sub> activity (9.0 and 8.2  $\mu$ M respectively) against the chloroquine-resistant strain K1 of *P. falciparum*, whereas **1** and **2** are showed only some moderate activity against other parasites, IC<sub>50</sub> = 59 and 51  $\mu$ M respectively against *Trypanosoma brucei rhodesiense* STIB 900 and IC<sub>50</sub> = 28  $\mu$ M for **2** against *Leishmania donovani* MHOM-ET-67/L82. The balgacyclamides A and B also displayed selectivity in respect to their cytotoxicity to the L6 rat myoblast cell line, for which no activity was detected up to 150  $\mu$ M. These results are in agreement with previous antiparasite evaluations of cyclamide compounds. The venturamides A and B display IC<sub>50</sub> of 8.2 and 5.2  $\mu$ M against *P. falciparum*<sup>8</sup>, while the aerucyclamides A, B, C and D display IC<sub>50</sub> of 5.0, 0.7, 2.3 and 6.3  $\mu$ M respectively.<sup>9</sup> It is interesting to note that opening of the oxazoline ring in **1** did not result in significant lower bioactivity.

In conclusion, we have reported the isolation and structure determination of the balgacyclamides A (**1**), B (**2**) and C (**3**) from *Microcystis aeruginosa* EAWAG 251.

Balgacyclamides A (**1**) and B (**2**) were evaluated for their antiparasite activity and displayed micromolar IC<sub>50</sub> activity against *P. falciparum* with a good selectivity compared to their cytotoxicity.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** NMR spectra were acquired on a Bruker Avance 500, a Bruker DRX-600 and a Bruker Avance II 800, all equipped with cryoprobes. The spectra were referred to residual solvent proton and carbon signals ( $\delta_{\text{H}}$  2.50,  $\delta_{\text{C}}$  39.5 for DMSO- *d*<sub>6</sub> and  $\delta_{\text{H}}$  7.26,  $\delta_{\text{C}}$  77.2 for CDCl<sub>3</sub>). Accurate mass ESI spectra were recorded on a Micromass (ESI) Q-TOF Ultima API. HPLC purification and analyses were performed on a Dionex HPLC system equipped with a P680 pump, an ASI-100 automated sample injector, a TCC-100 thermostated column compartment, a PDA-100 photodiode array detector, a Foxy Jr. fraction collector, and a MSQ-ESI mass spectrometric detector. The determination of the Ile enantiomers was carried out on a GC-FID instrument (Thermo Focus with a Thermo AS 3000 autosampler).

**Sources and Cultivation of Cyanobacteria.** *M. aeruginosa* EAWAG 251 was obtained from the Eawag (Swiss Federal Institute of Aquatic Science and Technology) Culture Collection of Cyanobacteria, currently at the University of Basel. This strain is identical as the *M. aeruginosa* PCC7820 from the Pasteur Culture Collection of Cyanobacteria, Paris, France. The strain was

grown in a 20 L batch reactor (Zehnder medium<sup>15</sup>) with continuous aeration and a light/dark cycle of 12:12 h. The biomass was isolated by centrifugation and subsequently kept frozen until extraction.

**Extraction and Isolation.** The biomass was extracted first with 100% MeOH and then twice with 60% MeOH. The extract was separated from the biomass by centrifugation. MeOH was removed from the combined extracts by evaporation under reduced pressure and the mixture was then dried by lyophilization. The resulting powder was dissolved in 80% MeOH and centrifuged to remove remaining particles. The solutions were then sequentially loaded to a C<sub>18</sub> SPE column (Supelco Discovery DSC-18, 10 g, conditioned with 10% aqueous MeOH), and eluted with 30%, 60%, 80%, and 100% aqueous MeOH solutions. MeOH was removed from the combined extract by evaporation under reduced pressure and the mixture was then dried by lyophilization. The resulting oil from the 80% MeOH fraction was redissolved in 80% MeOH, and the compounds were isolated using multiple C<sub>18</sub> RP-HPLC runs (Phenomenex Gemini C<sub>18</sub> 5  $\mu$ m; 150  $\times$  10 mm). The gradient MeOH/H<sub>2</sub>O at a flow of 5 mL/min was 35% MeOH to 100% over 30 min. The column was then washed for 10 min with 100% MeOH and stabilized for the next cycle in 10 min with 35% MeOH. MeOH was removed under reduced pressure and the compounds were then dried with a stream of N<sub>2</sub>. Balgacyclamides A, B and C eluted at 17.4, 13.2 and 14.3 min respectively. Balgacyclamide B and C were further purified by HPLC using multiple runs on an analytical column C<sub>18</sub> RP-HPLC (Phenomenex Gemini C<sub>18</sub> 5  $\mu$ m; 150  $\times$  4.6 mm). The gradient CH<sub>3</sub>CN/H<sub>2</sub>O at a flow of 1 mL/min was 30% CH<sub>3</sub>CN to 100% over 60 min. The column was then washed for 10 min with 100% CH<sub>3</sub>CN and stabilized for the next cycle in 10 min with 30% CH<sub>3</sub>CN. Balgacyclamide B and C eluted at 10.0 and 11.2 min respectively. The compounds were dried as described above. The isolation yields of the balgacyclamides A (**1**), B (**2**) and C (**3**) were 0.55 mg, 0.15 mg and 0.80 mg per 15 liters of culture, respectively.

**Balgacyclamide A (1):** colorless, amorphous solid; UV (CH<sub>3</sub>CN/ water, 55.5/45.5)  $\lambda_{\text{max}}$  234 nm; <sup>1</sup>H and <sup>13</sup>C NMR data (DMSO-*d*<sub>6</sub>), see Table 1; <sup>1</sup>H and <sup>13</sup>C NMR data (CDCl<sub>3</sub>), see supp. info.; HRESI-QqTof-MS *m/z* [M + H]<sup>+</sup> 533.2552 (calcd for C<sub>25</sub>H<sub>37</sub>N<sub>6</sub>O<sub>5</sub>S, 533.2546).

**Balgacyclamide B (2):** colorless, amorphous solid; UV (CH<sub>3</sub>CN/ water, 42/58)  $\lambda_{\text{max}}$  236 nm; <sup>1</sup>H and <sup>13</sup>C NMR data (DMSO-*d*<sub>6</sub>), see Table 2; HRESI-QqTof-MS *m/z* [M + H]<sup>+</sup> 551.2657 (calcd for C<sub>25</sub>H<sub>39</sub>N<sub>6</sub>O<sub>6</sub>S, 551.2652).

**Balgacyclamide C (3):** colorless, amorphous solid; UV (CH<sub>3</sub>CN/ water, 43/57)  $\lambda_{\text{max}}$  239 nm; <sup>1</sup>H and <sup>13</sup>C NMR data (DMSO-*d*<sub>6</sub>), see Table 3; HRESI-QqTof-MS *m/z* [M + H]<sup>+</sup> 585.2512 (calcd for C<sub>28</sub>H<sub>37</sub>N<sub>6</sub>O<sub>6</sub>S, 585.2495).

**Ozonolysis of compounds 1–3 :** Each compound (0.1 mg, 0.2  $\mu$ mol) was dissolved in 0.3 mL of dry CH<sub>2</sub>Cl<sub>2</sub>, ozonized at room temperature for 5 min and the solvent was removed under a stream of N<sub>2</sub>.

**Hydrolysis of compounds 1–3:** Each ozonized compound was hydrolyzed in a closed microwave tube by adding 0.5 mL of freshly prepared 6 N HCl at 108°C for 20 h. The solvent was concentrated by a stream of N<sub>2</sub> and removed by lyophilization and then the residue was dissolved in 0.06 mL of H<sub>2</sub>O.

**Marfey's Analysis of compounds 1–3:** Half of the solution of the hydrolysed compounds was treated with 0.03 mL of a 1% *N*-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA) solution in acetone and 0.03 mL of 6% Et<sub>3</sub>N in H<sub>2</sub>O at 50°C for 1 h in a closed HPLC vial. The reaction was quenched with 0.03 mL of 5% acetic acid and the solvent was removed by lyophilisation. The residue was solved in 0.03 mL of MeOH and analyzed by RP-HPLC using an Agilent Zorbax SB-C18 (3.55  $\mu$ m 150 x 2.1 mm). The mobile phases were A, 5% acetic acid in H<sub>2</sub>O, and B, MeCN/MeOH (9:1). The column was stabilized for 20 min with 5% of B, then the gradient went from 5-50% in 50 min and the column was washed with 100% of B for 20 min. The flow was set at 0.25 mL/min and the temperature of the column oven at 50°C.

By comparison of the retention times of the derivatized commercially available amino acids the configuration can be determined except for isoleucine. The retention times (min) for the derivatized standard amino acids were: L-Ala 28.25; D-Ala 32.21; L-Val 35.89; D-Val 40.64; L-Phe 41.01; D-Phe 46.39; L-Thr 21.09; L-*allo*-Thr 22.22; D-*allo*-Thr 24.73; D-Thr 28.89; L-Ile 40.47; L-*allo*-Ile 40.45; D-Ile 47.38 and D-*allo*-Ile 47.46.

**TFA Derivatives and Chiral GC Analysis of 1, 2 and 3 :** 0.03 mL of the hydrolyzed compound solution was transferred to a GC vial and the solvent was evaporated. The residue was treated with 0.03 mL of trifluoroacetic acid anhydride at room temperature for 12 hours and quenched with 0.03 mL of MeOH. The configuration of isoleucine was determined after analyses on a chiral GC column (Permaabond-L-Chirasil-Val; 25 m, 0.25 mm, Marcherey-Nagel, Düren, Germany) with the following conditions: 2 min at 80°C, 80 to 180°C at rate of 8°C min<sup>-1</sup> and 10 min at 180°C with a split ratio 10:1, at a flow of 1mL/min and a FID detector at 250°C.



The four commercially available stereoisomers of isoleucine were derivatized with the same method as presented above and the retention times (min) were: D-*allo*-Ile 6.20, L-*allo*-Ile 6.35, D-Ile 6.39 and L-Ile 6.45.

**Configuration of compounds 1-3 :**

Balgacyclamide A (**1**) : from ozonolysis–hydrolysis–Marfey D-Ala, L-Thr (confirmed by a co-injection with L-*allo*-Thr), L-Val and D-Ile or D-*allo*-Ile and from ozonolysis–hydrolysis–TFA D-*allo*-Ile (confirmed by a co-injection with D-Ile).

Balgacyclamide B (**2**) : from ozonolysis–hydrolysis–Marfey D-Ala, L-Thr, L-Val and D-Ile or D-*allo*-Ile and from ozonolysis–hydrolysis–TFA D-*allo*-Ile (confirmed by a co-injection with D-Ile).

Balgacyclamide C (**3**) : from ozonolysis–hydrolysis–Marfey L-Thr, L-Phe and D-Ile or D-*allo*-Ile and from ozonolysis–hydrolysis–TFA D-*allo*-Ile (confirmed by a co-injection with D-Ile).

*Plasmodium falciparum*, *Trypanosoma b. rhodesiense* and Cytotoxicity in Vitro Assay were performed according to procedures previously described.<sup>9b</sup>

**Acknowledgment.** Part of this research was supported by the NCCR Chemical Biology, funded by the Swiss National Science Foundation.

**Supporting Information Available:** Copies of spectra and additional data are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

**Table 1:** NMR spectroscopic data (500MHz, DMSO-*d*<sub>6</sub>) for balgacyclamide A (**1**).

balgacyclamide A			
C/N no.	$\delta_c$	$\delta_H$ ( <i>J</i> in Hz)	HMBC <sup>a</sup>
1	168.9, qC		
2	72.4, CH	4.41, dd (6.8, 2.0)	1, 4, 5
3	79.3, CH	4.95, dq (6.5, 6.4)	1, 5
4	20.4, CH <sub>3</sub>	1.37, d (6.4)	2, 3
5	166.0, qC		
6	50.6, CH	4.48, ddd (9.0, 2.5, 2.0)	5, 7, 8'
7	30.8, CH	1.98, m	
8	18.1, CH <sub>3</sub>	0.62, d (6.9)	6, 7, 8'
8'	15.1, CH <sub>3</sub>	0.35, d (6.9)	6, 7, 8
NH (2)		7.35, d (9.0)	5, 9
9	169.7, qC		
10	72.7, CH	4.33, d (6.7)	9 or 13, 11, 12
11	81.1, CH	4.79, dq (6.7, 6.3)	9 or 13
12	21.0, CH <sub>3</sub>	1.43, d (6.3)	9 or 13, 10, 11
13	169.7, qC		
14	42.6, CH	4.73, dq (7.7, 6.9)	13, 15, 16
15	19.3, CH <sub>3</sub>	1.47, d (6.9)	14
NH (4)		8.30, d (7.7)	
16	159.1, qC		
17	148.0, qC		
18	124.6, CH	8.30, s	17, 19
19	169.7, qC		
20	54.0, CH	5.23, dd (8.4, 5.7)	19, 21, 22
21	40.4, CH	1.92, m	
22	14.2, CH <sub>3</sub>	0.93, d (6.8)	20, 21, 23
23a	25.1, CH <sub>2</sub>	1.44, m	
23b		1.04, m	21
24	11.1, CH <sub>3</sub>	0.88, dd (7.4, 7.4)	21, 23
NH (6)		8.21, d (8.4)	1

<sup>a</sup> HMBC correlations are given from proton(s) stated to the indicated carbon atom.

**Table 2** : NMR spectroscopic data (500MHz, DMSO-*d*<sub>6</sub>) for balgacyclamide B (**2**).

balgacyclamide B				
C/H no.	$\delta_C$	$\delta_H$ ( <i>J</i> in Hz)	HMBC <sup>a</sup>	ROESY
1	169.7, qC			
2	73.4, CH	4.34, dd (6.2, 1.6)	1, 4, 5	4
3	78.0, CH	4.84, dq (6.2, 6.2)	1, 2, 5	
4	20.0, CH <sub>3</sub>	1.29, d (6.2)	2, 3	
5	165.7, qC			
6	51.1, CH	4.43, ddd (8.6, 3.7, 1.6)	5, 7, 8, 8', 9	4, 8, 8'
7	30.3, CH	1.95, m	5, 6, 8, 8'	8, 8'
8	17.8, CH <sub>3</sub>	0.67, d (6.9)	6, 7, 8'	
8'	15.8, CH <sub>3</sub>	0.53, d (6.9)	6, 7, 8	
NH (2)		6.95, d (8.6)	5, 6, 9	6, 7, 8, 8', 10, 11
9	169.4, qC			
10	58.5, CH	4.23, dd (8.7, 3.5)	9, 11, 13	12
11	65.0, CH	4.19, m	12	12
12	20.0, CH <sub>3</sub>	1.07, d (6.3)	10, 11	
OH		4.76, d (5.6)	10, 11, 12	12
NH (3)		8.47, d (8.7)	10, 11, 13	10, 11, 12, 14, 15, OH, NH(2)
13	172.4, qC			
14	47.8, CH	4.80, m	13, 15, 16	
15	17.4, CH <sub>3</sub>	1.30, d (6.5)	13, 14	
NH (4)		8.22, d (7.9)	13, 14, 16	14, 15, NH(2)
16	159.6, qC			
17	147.9, qC			
18	123.6, CH	8.22, s	17, 19	
19	169.6, qC			
20	54.4, CH	5.13, dd (8.5, 5.5)	1, 19, 21, 22, 23	22, 23b
21	39.5, CH	1.91, m	19, 20, 22, 23, 24	24
22	14.1, CH <sub>3</sub>	0.90, d (6.8)	20, 21, 23	
23a	25.2, CH <sub>2</sub>	1.44, m	20, 21, 22, 24	
23b		1.11, m	20, 21, 22, 24	
24	10.9, CH <sub>3</sub>	0.88, dd (7.3, 7.3)	20, 21, 23	
NH (6)		8.43, d (8.5)	1, 20	2, 6, 20, 21, 23a, 24

<sup>a</sup> HMBC correlations are given from proton(s) stated to the indicated carbon atom.

**Table 3:** NMR spectroscopic data (500MHz, DMSO-*d*<sub>6</sub>) for balgacyclamide C (**3**).

balgacyclamide C				
C/H no.	$\delta_c$	$\delta_H$ ( <i>J</i> in Hz)	HMBC <sup>a</sup>	ROESY
1	169.0, qC			
2	73.8, CH	4.28, dd (7.6, 1.7)	1, 3, 4, 5	4
3	78.3, CH	4.88, dq (7.6, 6.4)	1, 2, 5	
4	20.3, CH <sub>3</sub>	1.36, d (6.4)	2, 3	
5	165.0, qC			
6	48.0, CH	4.76, m	5, 7, 8, 12	7a
7a	36.7, CH <sub>2</sub>	2.98, dd (13.6, 5.2)	5, 6, 8, 9/9'	
7b		2.86, dd (13.6, 6.0)	5, 6, 8, 9/9'	
8	135.4, qC			
9/9'	129.0, CH	7.01, d (7.4)	7, 9/9', 11	3, 6, 7b, OH
10/10'	127.4, CH	6.94, dd (7.4, 7.4)	8, 9/9', 10/10'	3, 6, 7b, OH
11	126.0, CH	6.82, t (7.4)	9/9'	
NH (2)		7.45, d (7.5)	12	9/9', 6, 13, 14
12	169.2 qC			
13	58.8, CH	4.19, dd (9.0, 4.5)	12, 14, 15	15
14	65.4, CH	4.09, m	12	15
15	19.8, CH <sub>3</sub>	0.92, d (6.3)	13, 14	
OH		4.91, d (5.4)	13, 14, 15	14, 15
NH (3)		7.81, d (9.0)	13, 16	9/9', 13, 14, 15, 17a, OH, NH(2)
16	169.6, qC			
17a	42.9, CH <sub>2</sub>	4.39, dd (15.3, 7.5)	16, 18	
17b		3.63, dd (15.3, 3.3)	16, 18	
NH (4)		8.26, dd (7.5, 3.3)		17a, 17b, NH(2), NH(3), NH(6)
18	159.9, qC			
19	147.5, qC			
20	123.7, CH	8.29, s	18, 19, 21	
21	168.6, qC			
22	54.1, CH	5.18, dd (8.1, 5.2)	21, 23, 24, 25	23, 26
23	39.8, CH	1.92, m		26
24	13.9, CH <sub>3</sub>	0.84, d (6.9)	22, 23, 25	
25a	25.0, CH <sub>2</sub>	1.45, m	22, 23, 24, 26	
25b		0.98, m	22, 23, 24	

26	11.2, CH <sub>3</sub>	0.87, dd (7.4, 7.4)	23, 25	
NH (6)		7.98, d (8.2)	1, 22	2, 22, 23, 24, 25a

---

<sup>a</sup> HMBC correlations are given from proton(s) stated to the indicated carbon atom.

**Table 4.** Biological evaluation of balgacyclamides A (**1**) and B (**2**) against *Plasmodium falciparum* K1, *Trypanosoma brucei rhodesiense* STIB 900, *Trypanosoma cruzi* Tulahuen C4, *Leishmania donovani* MHOM-ET-67/L82 and rat myoblast cells L6 (IC<sub>50</sub> values are reported  $\mu$ M as concentrations).

	<i>P.f.</i> <sup>a</sup>	<i>T. b. rhod.</i> <sup>b</sup>	<i>T. cruzi</i> <sup>c</sup>	<i>L. donovani</i> <sup>d</sup>	L6 <sup>e</sup>
<b>1</b>	9.0	59	>150	>150	>150
<b>2</b>	8.2	51	>150	28	>150

<sup>a</sup> *Plasmodium falciparum* K1

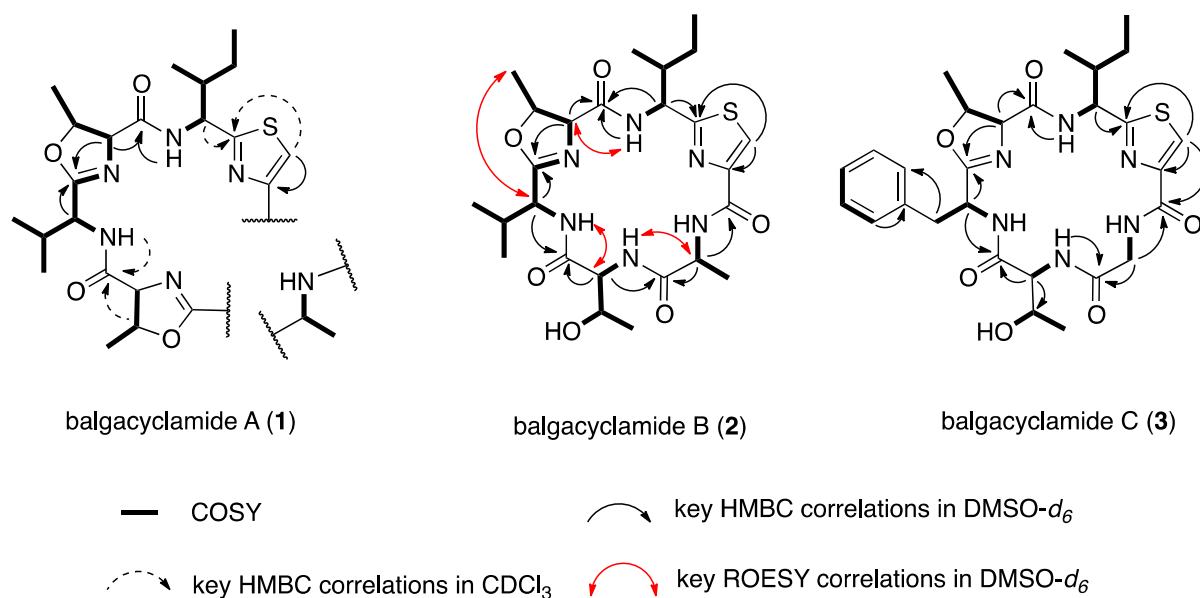
<sup>b</sup> *Trypanosoma brucei rhodesiense* STIB 900

<sup>c</sup> *Trypanosoma cruzi* Tulahuen C4

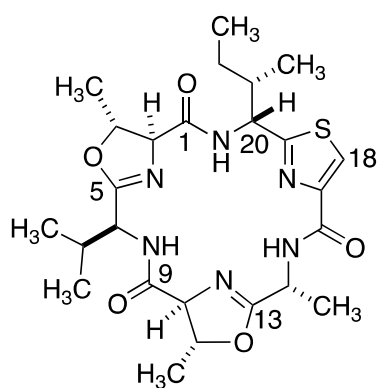
<sup>d</sup> *Leishmania donovani* MHOM-ET-67/L82

<sup>e</sup> Rat myoblast L6 cells

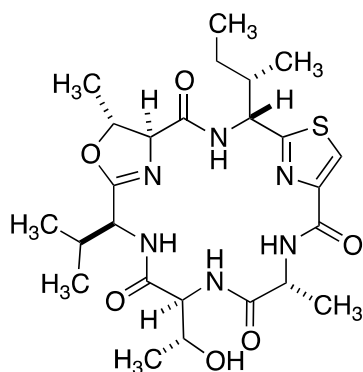
**Figure 1:** Key COSY, ROESY and HMBC correlations in balgacyclamides A (**1**), B (**2**) and C (**3**). Note the observed long-range *J* coupling between H-2 and H-6 in both balgacyclamides A (**1**) and B (**2**).



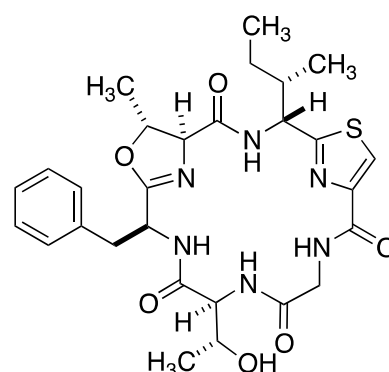




Balgacyclamide A (1)



Balgacyclamide B (2)



Balgacyclamide C (3)

## References

- (1) Reviews: (a) Giordanetto, F.; Kihlberg J. *J. Med. Chem.* **2013**, doi: 10.1021/jm400887j. (b) Katoh, T.; Goto, Y.; Reza, M. S.; Suga, H. *Chem Commun* **2011**, 47, 9946–9958. (c) Vlieghe, P.; Lisowski, V.; Martinez, J.; Khrestchatisky, M. *Drug Discovery Today* **2010**, 15, 40–56. (d) Purcell, A. W.; McCluskey, J.; Rossjohn, J. *Nat. Rev. Drug. Discov.* **2007**, 6, 404–414. (e) Kahne, D.; Leimkuhler, C.; Wei, L.; Walsh, C. *Chem. Rev.* **2005**, 105, 425–448.
- (2) Arnison, P. G.; Bibb, M. J.; Bierbaum, G.; Bowers, A. A.; Bugni, T. S.; Bulaj, G.; Camarero, J. A.; Campopiano, D. J.; Challis, G. L.; Clardy, J.; Cotter, P. D.; Craik, D. J.; Dawson, M.; Dittmann, E.; Donadio, S.; Dorrestein, P. C.; Entian, K. D.; Fischbach, M. A.; Garavelli, J. S.; Göransson, U.; Gruber, C. W.; Haft, D. H.; Hemscheidt, T. K.; Hertweck, C.; Hill, C.; Horswill, A. R.; Jaspars, M.; Kelly, W. L.; Klinman, J. P.; Kuipers, O. P.; Link, A. J.; Liu, W.; Marahiel, M. A.; Mitchell, D. A.; Moll, G. N.; Moore, B. S.; Müller, R.; Nair, S. K.; Nes, I. F.; Norris, G. E.; Olivera, B. M.; Onaka, H.; Patchett, M. L.; Piel, J.; Reaney, M. J.; Rebuffat, S.; Ross, R. P.; Sahl, H. G.; Schmidt, E. W.; Selsted, M. E.; Severinov, K.; Shen, B.; Sivonen, K.; Smith, L.; Stein, T.; Süßmuth, R. D.; Tagg, J. R.; Tang, G. L.; Truman, A. W.; Vederas, J. C.; Walsh, C. T.; Walton, J. D.; Wenzel, S. C.; Willey, J. M.; van der Donk, W. A. *Nat. Prod. Rep.* **2013**, 30, 108–160.
- (3) (a) Donia, M. S.; Ravel, J.; Schmidt, E. W. *Nat. Chem. Biol.* **2008**, 4, 341–343. (b) Sivonen, K.; Leikoski, N.; Fewer, D. P.; Jokela, J. *Appl. Microbiol. Biotechnol.* **2010**, 86, 1213–1225.
- (4) (a) Donia, M. S.; Hathaway, B. J.; Sudek, S.; Haygood, M. G.; Rosovitz, M. J.; Ravel, J.; Schmidt, E. W. *Nat. Chem. Biol.* **2006**, 2, 729–735. (b) Schmidt, E. W.; Nelson, J. T.; Rasko, D. A.; Sudek, S.; Eisen, J. A.; Haygood, M. G.; Ravel, J. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, 102, 7315–7320. (c) Houssen, W. E.; Koehnke, J.; Zollman, D.; Vendome, J.; Raab, A.; Smith, M. C. M.; Naismith, J. H.; Jaspars, M. *Chem. Bio. Chem.* **2012**, 13, 2683–2689.

- (5) Reviews (a) Gademann, K. *Chimia* **2011**, *65*, 416-419. (b) Gademann, K.; Sieber S. *Chimia* **2011**, *65*, 835-838. (c) Gademann, K.; Portmann, C. *Curr. Org. Chem.* **2008**, *12*, 326-341. See, for example: (d) Hambley, T. W.; Hawkins, C. J.; Lavin, M. F.; Van Den Brenk, A.; Watters, D. J. *Tetrahedron* **1992**, *48*, 341-348. (e) Prinsep, M. R.; Moore, R. E.; Levine, I. A.; Patterson, G. M. L. *J. Nat. Prod.* **1992**, *55*, 140-142. (f) Admi, V.; Afek, U.; Carmeli, S. *J. Nat. Prod.* **1996**, *59*, 396-399. (g) Banker, R.; Carmeli, S. *J. Nat. Prod.* **1998**, *61*, 1248-1251. (h) Ishida, K.; Nakagawa, H.; Murakami, M. *J. Nat. Prod.* **2000**, *63*, 1315-1317. (i) Ploutno, A.; Carmeli, S. *Tetrahedron* **2002**, *58*, 9949-9957. (j) Ziemert, N.; Ishida, K.; Quillardet, P.; Bouchier, C.; Hertweck, C.; Tandeau de Marsac, N.; Dittmann, E. *Appl. Environ. Microbiol.* **2008**, *74*, 1791. (k) Leikoski, N.; Fewer, D. P.; Jokela, J.; Alakoski, P.; Wahlsten, M.; Sivonen, K. *PLoS ONE* **2012**, *7*, e43002.
- (6) (a) Todorova, A.; Jüttner, F. *Phycologia* **1996**, *35*, 183-188. (b) Todorova, A. K.; Jüttner, F.; Linden, A.; Plüss, T.; von Philipsborn, W. *J. Org. Chem.* **1995**, *60*, 7891-7895. (c) Jüttner, F.; Todorova, A. K.; Walch, N.; von Philipsborn, W. *Phytochemistry* **2001**, *57*, 613-619. (d) Koodkaew, I.; Matsuyama, S.; Sunohara, Y.; Matsumoto, H. *Tetrahedron Lett.* **2012**, *53*, 977-979. (e) Koodkaew, I.; Sunohara, Y.; Matsuyama, S.; Matsumoto, H. *Plant Physiol. Biochem.* **2012**, *58*, 23-28.
- (7) Ogino, J.; Moore, R. E.; Patterson, G. M. L.; Smith, C. D. *J. Nat. Prod.* **1996**, *59*, 581-586.
- (8) Linington, R. G.; González, J.; Ureña, L. D.; Romero, L. I.; Ortega-Barría, E.; Gerwick, W. H. *J. Nat. Prod.* **2007**, *70*, 397-401.
- (9) (a) Portmann, C.; Blom, J. F.; Gademann, K.; Jüttner, F. *J. Nat. Prod.* **2008**, *71*, 1193-1196. (b) Portmann, C.; Blom, J. F.; Kaiser, M.; Brun, R.; Jüttner, F.; Gademann, K. *J. Nat. Prod.* **2008**, *71*, 1891-1896.
- (10) (a) Peña, S.; Scarone, L.; Manta, E.; Serra, G. *Tetrahedron Lett.* **2013**, *54*, 2806-2808. (b) Peña, S.; Scarone, L.; Manta, E.; Stewart, L.; Yardley, V.; Croft, S.; Serra, G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4994-4997. (c) Peña, S.; Scarone, L.; Medeiros, E.; Manta, E.; Comini, M.; Serra, G. *MedChemComm* **2012**, *3*, 1443-1448.
- (11) Foster, M. P.; Concepcion, G. P.; Caraan, G. B.; Ireland, C. M. *J. Org. Chem.* **1992**, *57*, 6671-6675.
- (12) (a) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1354-1359. (b) Sone, H.; Kigoshi, H.; Yamada, K. *Tetrahedron* **1997**, *53*, 8149-8154. (c) Hambley, T. W.; Hawkins, C. J.; Lavin, M. F.; Van Den Brenk, A.; Watters, D. J. *Tetrahedron* **1992**, *48*, 341-348.

- (13) McDonald, L. A.; Ireland, C. M. *J. Nat. Prod.* **1992**, *55*, 376.
- (14) (a) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591. (b) Hess, S.; Gustafson, K. R.; Milanowski, D. J.; Alvira, E.; Lipton, M. A.; Pannell, L. K. *J. Chromatogr. A* **2004**, *1035*, 211–219.
- (15) Staub, R. *Schweiz. Z. Hydrol.* **1961**, *23*, 82–198.